

CARBON DIOXIDE EUTHANASIA OF RODENTS

I. Purpose/Scope

To the greatest extent possible, animals being euthanized should not experience pain, fear, or other significant stress prior to their death. Animals must be euthanized by humane methods as described in the 2007 AVMA Panel on Euthanasia, which states that “compressed CO₂ in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other means such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable.” Neonatal rodents are unusually resistant to hypoxia and to the inhalant anesthetics often used as euthanizing agents in adult rodents.

II. Sources

2007 AVMA Panel on Euthanasia
American College of Laboratory Animal Medicine Report of the ACLAM Task Force on Rodent Euthanasia
Guide for the Care and Use of Laboratory Animals (1996)
Pritchett, K., D. Corrow, J. Stockwell, and A. Smith. 2005. Euthanasia of Neonatal Mice with Carbon Dioxide. *Comparative Medicine* 55(3): 275-281.
Pritchett-Corning, K. 2009. Euthanasia of Neonatal Rats with Carbon Dioxide. *JAALAS* 48(1): 23-27.

III. Policy

Carbon dioxide is the preferred method of euthanasia for rodents. Exposure to high concentrations of CO₂ has an initial rapid depressant and anesthetic effect followed by death through asphyxiation. Unconsciousness is induced most rapidly by exposing animals to a CO₂ concentration of 70% or more. Any rodent used for research may be euthanized by CO₂ by following the guidance described below. Examples include mice of the genus *Mus*, rats of the genus *Rattus*, in addition to hamsters, gerbils, and other laboratory rodents.

A. Administering CO₂

1. All personnel administering CO₂ to rodents must be properly trained. All Principal Investigators must assure that their research staff is duly trained and adhere to animal care and use protocols, policies, and guidelines.
2. Compressed gas is the only acceptable source of CO₂ for euthanizing rodents.
3. Euthanasia chambers should be constructed of clear material to allow visualization of the animals, and must be kept clean to minimize odors that might distress animals subsequently euthanized. All animals in the chamber must be able to make normal postural adjustments and should not be overcrowded. Gas

should be delivered in a predictable and controllable fashion, at a low-flow rate.

4. Euthanasia should occur in a procedure room or laboratory, away from other rodent housing. Euthanasia should never be performed in the animal room.
5. The chamber should be kept clean to minimize odors that might cause distress to animals subsequently euthanized.
6. Several animals may be euthanized simultaneously, but mixing or combining of animals from multiple cages, into a single cage, is prohibited.
7. Do not “pre-fill” the euthanasia chamber with CO₂. Start with room air then slowly fill the chamber with CO₂ over several minutes. CO₂ is heavier than room air, thus the chamber may need to be “purged” between groups of cages.
8. CO₂ first renders the animal anesthetized and then, with adequate exposure time, will result in death by CO₂ narcosis. Animals should be left for additional time within the euthanasia chamber, after spontaneous movements have ceased, with CO₂ continuing to flow. Preferably, a secondary method of euthanasia should be performed.
9. Carcass bags must be identified with the date, PI, and protocol number before being placed in the freezer.

Resistance to hypoxia results in a prolonged time to unconsciousness when carbon dioxide inhalation is used as a euthanasia agent in neonatal rodents. The duration of exposure to carbon dioxide varies with the age of the neonate compared with adult rodents.

<u>AGE</u>	<u>Mice</u>	<u>Rats</u>
Non-haired pups		
0-6 days	60 minutes	40 minutes
Haired pups, eyes closed		
7-13 days	20 minutes	20 minutes
Haired pups, eyes open, preweaning		
14-20 days	10 minutes	10 minutes
Weanlings and adults		
21+ days	5 minutes	5 minutes

Other methods that may be used for euthanasia of neonatal rodents includes: injection of chemical anesthetics (e.g., pentobarbital), decapitation, or cervical dislocation. Any deviation from this guideline concerning euthanasia of neonatal rodents will be considered and reviewed by the IACUC on a case-by-case basis.

B. Confirming Death

Since the anesthetic effects of CO₂ are reversible, animals that are prematurely removed from the chamber prior to death can recover. Furthermore, death should be confirmed by personnel who have been specifically trained to recognize cessation of vital signs in rodents. Therefore, all animals being euthanized with CO₂ overdose must also receive a confirmatory method of euthanasia to ensure death. These

confirmatory methods, to be performed after CO₂ overdose, include exsanguination, decapitation, cervical dislocation, bilateral thoracotomy, or at least 50% additional time in the euthanasia chamber filled with 100% CO₂. Death of the animal must be ensured prior to disposal of the rodent carcass. Failure to confirm death of euthanized rodents is a significant non-compliance, reportable to the appropriate regulatory and accrediting agencies.

C. Exemptions

Methods of euthanasia used will be consistent with the recommendations of the 2007 AVMA Panel on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator. Any departures from these guidelines will require the PI to provide strong scientific and clinical justifications for the exemption. These justifications must be submitted to the IACUC in the Animal Use Protocol and will be reviewed by the IACUC in consultation with the attending veterinarian.